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Research article

ERK activation in the prefrontal cortex by acute apomorphine and apomorphine conditioned contextual stimuli

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ABSTRACT

The activation of extracellular signal-regulated kinase protein (ERK) has been linked to the adaptive responses to environmental changes and memory. The aim of this study was to measure ERK activation in primary dopamine projection areas namely, the prefrontal cortex and the nucleus accumbens, following a conditioned dopaminergic drug response. Initially, the effect of unconditioned apomorphine (2.0 mg/kg) administration on ERK activation was measured and the results showed an increase in ERK for both brain regions. Subsequently, two experiments were conducted to assess ERK activation in these two areas following apomorphine conditioned contextual stimuli. In experiment 1, rats received 5 daily injections of 2.0 mg/kg apomorphine or vehicle immediately prior to placement in an open-field. After a withdrawal period of two days, a conditioning test was conducted, in which rats received a 30 min non-drug test. Immediately after completion of the test, an immunohistochemical protocol was carried out to measure ERK activation. In experiment 2, a similar test protocol was performed except that the treatments were administered 30 min following open-field tests (post-trial experiment). The results showed that the repeated apomorphine treatments given prior to testing induced conditioned effects. An increase in ERK activation was seen in the prefrontal cortex but not in the nucleus accumbens. There was no conditioning response observed in the post-trial experiment and no differential ERK activation. These observations implicate the prefrontal cortex in the associative neuro-adaptive changes induced by dopaminergic stimulation.

1. Introduction

With repeated treatments, the behavioral effects of psychostimulant drugs such as apomorphine are potentiated (Damianopoulos and Carey, 1993; Mattingly et al., 1997; Rowlett et al., 1997; de Matos et al., 2010; Coelho et al., 2011; Mattingly et al., 2001; Braga et al., 2009c; Sanguedo et al., 2014). In that psychostimulant behavioral sensitization effects persist long after drug withdrawal, they are considered to represent an enduring drug induced alteration of the nervous system. Furthermore, this sensitization phenomenon has been repeatedly demonstrated for a number of psychostimulant drugs with addictive properties and indeed sensitization has been considered an important contributor to the addictive liability of these drugs (Robinson and Berridge, 1993; Stewart and Badiani, 1993; Carey and Damianopoulos, 2006).

It is now well established that dopamine systems are strongly implicated in psychostimulant conditioning and sensitization processes. The involvement of dopamine systems in this type of conditioning has been demonstrated frequently in drug conditioning studies in which dopaminergic drugs are used as unconditioned stimuli to induce conditioned drug effects. Repeated pairings of the drug treatments with placement in a specific test environment commonly results in context specific drug conditioning and sensitization effects (Mazurski and Beninger, 1991; Carey and Gui, 1998; Bloise et al., 2007; Braga et al., 2009a,b). In several previous reports, we have shown that repeated high dose apomorphine treatments induce hyper locomotion and that this behavioral response undergoes sensitization that is context specific (Bloise et al., 2007; Braga et al., 2009a,b; Dias et al., 2010; de Matos et al., 2010) and in addition, generates conditioned locomotor stimulant effects (Braga et al., 2009a,b; Dias et al., 2010; de Matos et al., 2010).

An important pharmacological characteristic of apomorphine is that

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its effect upon the dopamine system can be either inhibitory or facilitatory depending upon the administrated dose. Low doses (< 0.1 mg/kg) of apomorphine preferentially stimulate dopamine auto-receptors and thus inhibit dopamine neurons (Aghajanian and Bunney, 1973; DiChiara et al., 1977; Missale et al., 1998). When dose levels are increased (> 0.2 mg/kg), apomorphine increases post-synaptic dopamine receptor stimulation and functions as a dopamine agonist and psychostimulant drug (Mattingly et al., 1988a,b; Rowlett et al., 1997). This apomorphine functional duality when acting on the dopamine system means that it can be considered as either a pro-dopamine or an anti-dopamine treatment depending upon dosage.

In the search for the identity of cellular and molecular changes in the brain areas pertinent to the sensitization effects of repeated psychostimulant drug treatments, ERK has generated significant interest (Adams and Sweatt, 2002; Valjent et al., 2004, 2005; Radwanska et al., 2005; Girault et al., 2007; Lu et al., 2006; Shiflett and Balleine, 2011). Furthermore, psychostimulant drugs such as cocaine produce an increased ERK response in striatal dopaminergic projection areas (Valjent et al., 2005; DiRocco et al., 2009; Janes et al., 2009; Fricks-Gleason and Marshall, 2011), including the nucleus accumbens (Marin et al., 2009), frontal cortex (Li et al., 2008) and amygdala (Radwanska et al., 2005). In line with these findings, we recently reported (Sanguedo et al., 2014) that apomorphine sensitization selectively potentiates the apomorphine induced ERK response in the prefrontal cortex. In that we have also shown that apomorphine sensitization is context specific (de Matos et al., 2010), our ERK sensitization findings suggested that the prefrontal cortex is involved in associative drug responses. In the present investigation, we initially induced apomorphine sensitization and then conducted a non-drug test for conditioned effects. Following the conditioning tests, we measured ERK in the prefrontal cortex as well as another major dopamine projection site namely the nucleus accumbens. The present report details the changes in ERK associated with conditioned apomorphine behavior.

2. Methods

2.1. Subjects

Male Wistar albino rats provided by the State University of North Fluminense, initially weighing 250–300 g were housed in individual plastic cages (25 × 18 × 17 cm) until the end of the experiment. Food and water were freely available at all times. The vivarium was maintained at a constant temperature (22 + 2 °C), humidity controlled and a 12/12 h light/dark cycle (lights on at 07:00 h and off at 19:00 h). All experiment occurred between 9:00 and 14:00 h. For 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. All experiments were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Apomorphine-HCl (Sigma, St. Louis, MO, USA) was dissolved in 0.1% ascorbate/saline solution at a concentration of 2.0 mg/ml and injected subcutaneously in the nape of the neck at a dose of 2.0 mg/kg. All solutions were freshly prepared and administered in a volume of 1 ml/kg.

2.3. Apparatus and environmental context of drug treatment

The behavioral measurements were conducted in a black open field chamber (60 × 60 × 45 cm). A closed-circuit video camera (IKEGAMI, model ICD-49), mounted 60 cm above the arena was used to record behavioral data. Locomotion, measured as distance traveled (m), was automatically analyzed by EthoVision software (Noldus, The Netherlands). The complete test procedure was conducted automatically without the presence of the experimenter in the test room. All behavioral testing was conducted under dim red light to avoid the possible aversive quality of white light and to enhance the contrast between the white subject and dark background of the test chamber. Testing under red light conditions is less stressful and also favors locomotor activation as the rats are transferred from the ambient light of the vivarium to the red light of the testing room (Nasello et al., 1988). Masking noise was provided by a fan located in the experimental room that was turned on immediately prior to placing the animal in the experimental arena and turned off upon removal of the animal from the experimental arena (i.e. test chamber).

2.4. Experimental protocol

2.4.1. Apomorphine time course experiment

In order to assess the magnitude and time course of the ERK induced by the apomorphine treatment (2.0 mg/kg) used in the present study, we initially measured the ERK activation following the 2.0 mg/kg apomorphine acute treatment. Eight groups of rats were given either vehicle (4 groups, n = 4 for each group) or 2.0 mg/kg apomorphine (4 groups; n = 4 for each group) and returned to their home-cage and either 5, 15, 30 or 60 min later were euthanized and ERK measurements were made in the prefrontal cortex and the nucleus accumbens.

2.4.2. Conditioning experiment 1

Initially all rats received three 30 min habituation sessions conducted on consecutive days. The habituation protocol was conducted so that a stable baseline of locomotor behavior could be established prior to the start of the drug treatments. The animals were administered vehicle, placed in the experimental arena and locomotor activity was measured. After the third habituation test session, the animals were assigned to groups equated on locomotor activity over the three test sessions ($p > 0.05$). There were two treatment groups: an apomorphine group and a vehicle treatment group. In the apomorphine group, rats received injections of 2.0 mg/kg apomorphine (APO-2.0; n = 6) immediately before placement in the test arena and vehicle administration 30 min after the end of the arena test. The vehicle group (VEH; n = 6) was treated in the same way as the apomorphine group except that the animals received only injections of vehicle. These treatments were administered for 5 consecutive days, one trial per day and served as the conditioning induction phase. The induction phase was designed to establish an apomorphine sensitized response effect selectively in the apomorphine treatment group. After a period of 2 days without injections or behavioral testing (withdrawal period), in order to insure an effective drug washout for the short duration acting apomorphine, the conditioning test was performed in which the animals received vehicle prior to being placed into the test environment and locomotion was recorded for 30 min. Immediately after the end of the conditioning test, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg I.P.), perfused transcardially and the brains removed and stored (as described in Section 2.5) for subsequent immunohistochemical phosphorylated-ERK-P analysis.

2.4.3. Conditioning experiment 2

This experiment was a replication of conditioning experiment 1 except that during the 5 day induction phase both the apomorphine (2.0 mg/kg) and the vehicle groups received vehicle prior to testing. Thirty min after completion of the arena tests, there was a post-trial treatment (P), in which the apomorphine group received apomorphine (APO-P, n = 6) and the vehicle group received vehicle (VEH-P, n = 4). In this experiment, the apomorphine group received the same apomorphine exposure as the apomorphine group in conditioning experiment 1, but not in association with the test arena. The assumption of conditioning experiment 1 was that an effect of the apomorphine treatment on the conditioning test was attributable to the association of the drug effect with the test arena cues for this experiment controlled